IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

*In re* application of:
S. KADOTA
Appl. No.: 10/825,585
Filed: April 16, 2004
For: **Agents for Treating Osteoporosis and Inhibiting Osteoclast Formation**

Art Unit: 1655
Examiner: M. FLOOD
Atty. Docket: 0804.001.0002
Confirmation No.: 8180
Customer No.: 43446

## DECLARATION UNDER 37 C.F.R.§1.132

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

1. My name is Chia-Chin Sheu and I am employed by the assignee of the above-identified patent application. I have been employed by the Assignee in the position of President of Simpson Biotech Co., Ltd. since Oct 1998.

2. I have a background in Bio-Chemistry & Analytical Chemistry / Master of Sci.

3. I understand that the claims of the above-identified patent application have been rejected over Koh et al., which uses ethylacetate in a process to produce a water extract of *Cordyceps sinensis*.

4. I performed the following experiments, which depict differences between a water extract of *Cordyceps sinensis* obtained using diethyl ether in the process and a water extract obtained using ethylacetate in the process.

**(A) Extraction procedure:**

(a) Applicants' invention (WE-sb)

1. heating and refluxing *Cordyceps sinensis* mycelium (10.00 g) in 300 ml diethyl ether for 2 hours,
2. heating and refluxing the residue of the mycelium in 300 ml methanol for 2 hours,
3. heating and refluxing the residue of the mycelium in 300 ml deionized water, heat and reflux for 2 hours,
4. centrifuging and filtrating the water extract, and reserving the supernatant,
5. performing nucleoside HPLC for the supernatant, and
6. freezing and drying the remaining supernatant for performing GPC HPLC.

The spectrum of nucleoside HPLC was shown in Figure S1 (A) and the spectrum of GPC HPLC was shown in Figure S2(A)-(C).

(b) Koh Reference (WE-koh)

1. extracting *Cordyceps sinensis* mycelium (10.00 g) in 300 ml ethylacetate,
2. heat extracting the residue of the mycelium in 300 ml methanol,
3. heat extracting the residue of the mycelium in 300 ml distilled water,
4. centrifuging and filtrating the hot water extract, and reserving the supernatant,
5. performing nucleoside HPLC for the supernatant, and
6. freezing drying the remaining supernatant for performing GPC HPLC.

The spectrum of nucleoside HPLC was shown in Figure S1 (B) and the spectrum of GPC HPLC was shown in Figure S2(D)~(F).

**(B) Analysis method:**

(a) nucleoside HPLC:

1. moving phase: PBS, methanol
2. column: Zorbax SB-C18, 4.6 × 250 mm
3. flow rate: 0.9 ml/ min
4. temperature: 30 ℃
5. injecting volume: 20 ul
6. wavelength: 260 nm

(b) GPC HPLC:

1. moving phase: deionized water, methanol
2. column: Waters, Ultrahydrogel, 7.8 × 300 mm,
   HSPgel AQ MB-H 6.0 × 150 mm

3. flow rate: 0.3 ml/ min
4. temperature: 30 ℃
5. injecting volume: 10 ul
6. serial connecting 3 detectors: PDA Detector for distribution of protein molecular weight, Conductivity Detector for charged substances, and RI Detector for polysaccharides

(c) Protein concentration determination: BCA kit
(d) Lipid determination based on Taiwan CNS standard method
(e) Carbohydrate determination
(f) Ash determination
(g) Reduced sugar determination: Antrone method

**(C) Results**

(a) Profile of HPLC:

The *Cordyceps sinensis* mycelium was cultured by liquid fermentation. Water extracts, WE-sb and WE-koh, extracted from the mycelium by various solvents were analyzed by HPLC. The profile of nucleoside HPLC was shown in Figure S1 and the profile of GPC HPLC was shown in Figure S2.

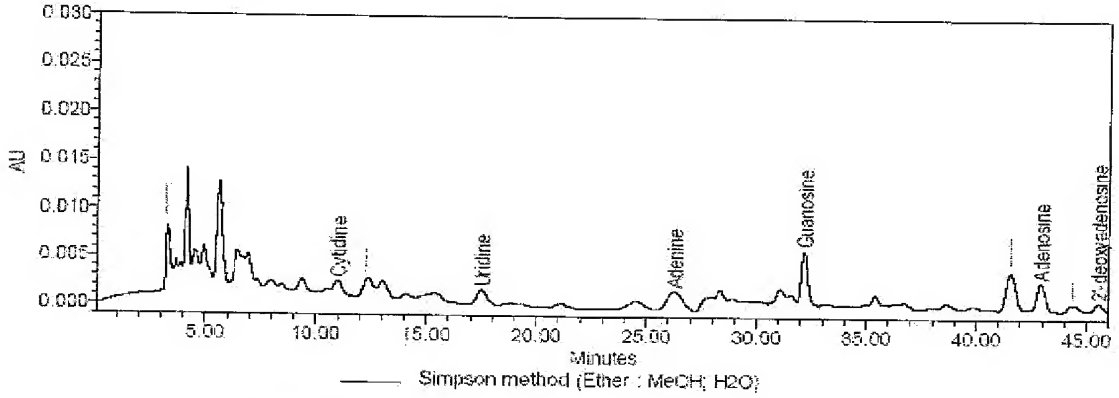
The profiles of WE-sb and WE-koh were compared by Photodiode Array Detector. It was found the nucleoside HPLC profile of WE-sb was not identical with that of WE-koh. The GPC HPLC profile of WE-sb was not identical with that of WE-koh, either. The different peaks between WE-sb and WE-koh are indicated by arrowheads.

Besides, the nucleoside content of WE-koh such as cytidine, uridine, adenine, guanosine, adenosine, and 2-deoxyadenosine was slight higher than WE-sb. The water extract content of WE-koh was lower than WE-sb.

(b) Content percentage:

| Analysis | WE-sb (Ether→MeOH→Hot Water) | WE-koh (EtOAc→MeOH→Hot Water) |
|---|---|---|
| **Nucleoside HPLC analysis (Figure S1(A) & S1(B))** | | |
| Nucleosides | | Slight higher than WE-sb |
| **GPC HPLC analysis (Figure S1(A)-(C) & S1(D)-(F))** | | |
| PDA Detector | Total area: higher than WE-koh | |
| Conductivity Detector | Charged substance: more than WE-koh | |
| RI Detector | | RT at 50 minutes, Peak Area: higher than WE-sb |
| **Content determination** | | |
| Protein (%) | 6.82 | 16.62 |
| Lipid (%) | 1.46 | 2.16 |
| Carbohydrate (%) | 83.18 | 70.28 |
| Ash (%) | 8.54 | 10.94 |
| Suger (%) | 53.9 | 61.4 |

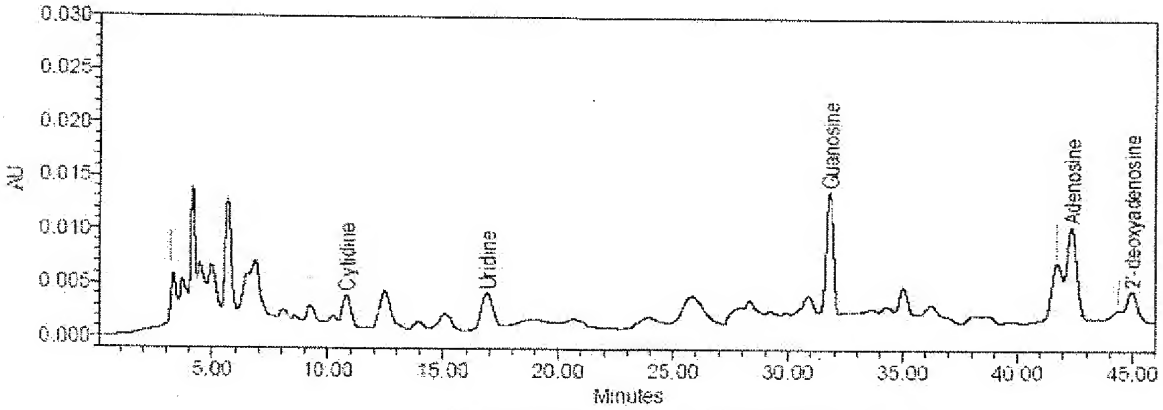
Figure S1 (A)




Peak Results

| | Name | RT | Area | Amount | Units |
|---|---|---|---|---|---|
| 1 | Cytidine | 10.996 | 24013 | 0.018 | mg/g |
| 2 | Uridine | 17.502 | 38290 | 0.021 | mg/g |
| 3 | Adenine | 26.273 | 62000 | 0.015 | mg/g |
| 4 | Guanosine | 32.160 | 101937 | 0.062 | mg/g |
| 5 | Adenosine | 42.928 | 72121 | 0.030 | mg/g |
| 6 | 2'-deoxyadenosine | 45.547 | 17954 | 0.008 | mg/g |

(B)




Peak Results

| | Name | RT | Area | Amount | Units |
|---|---|---|---|---|---|
| 1 | Cytidine | 10.808 | 69375 | 0.055 | mg/g |
| 2 | Uridine | 16.900 | 103926 | 0.056 | mg/g |
| 3 | Guanosine | 31.807 | 239601 | 0.149 | mg/g |
| 4 | Adenosine | 42.359 | 245999 | 0.103 | mg/g |
| 5 | 2'-deoxyadenosine | 44.977 | 58743 | 0.023 | mg/g |

Figure S2

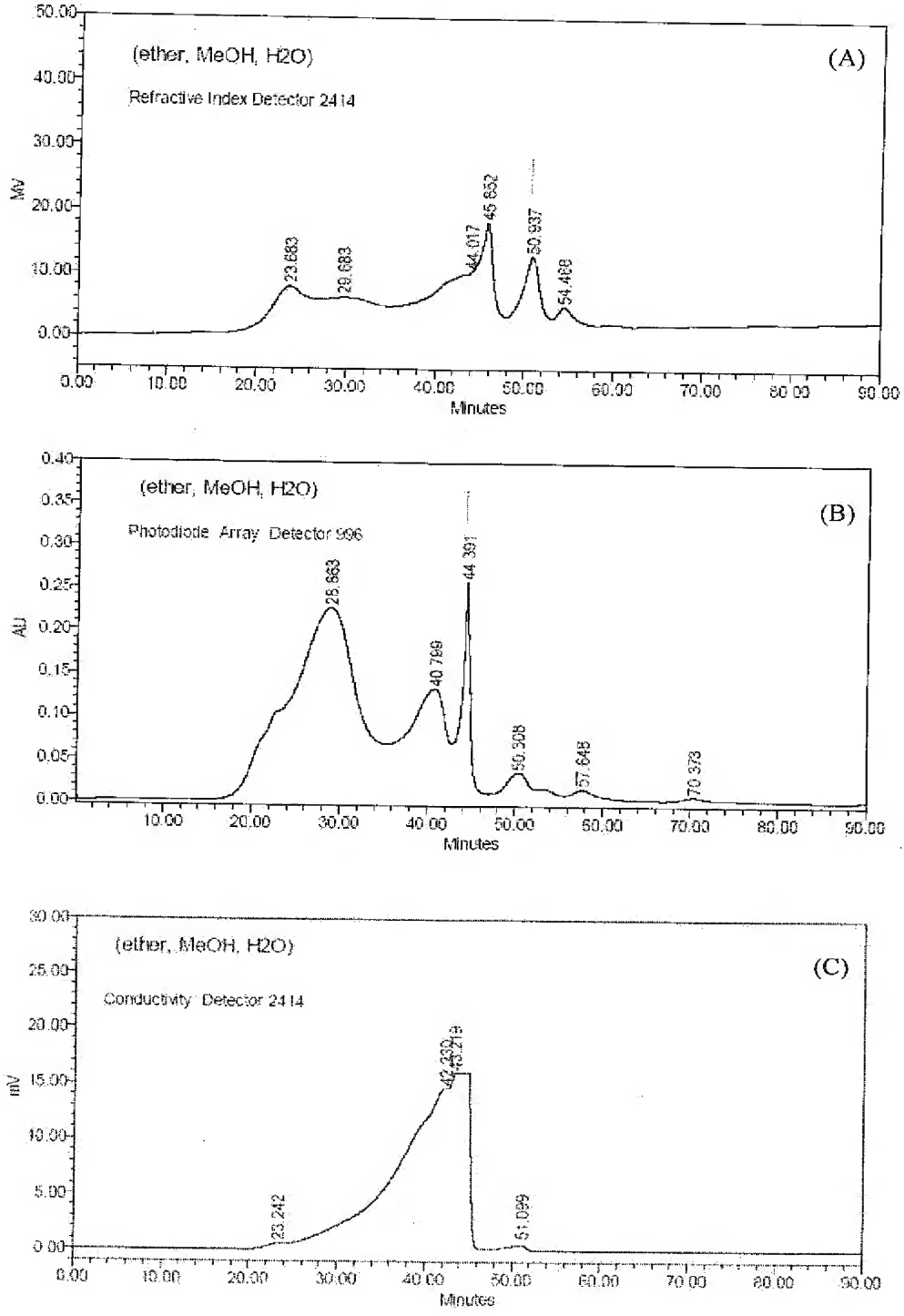
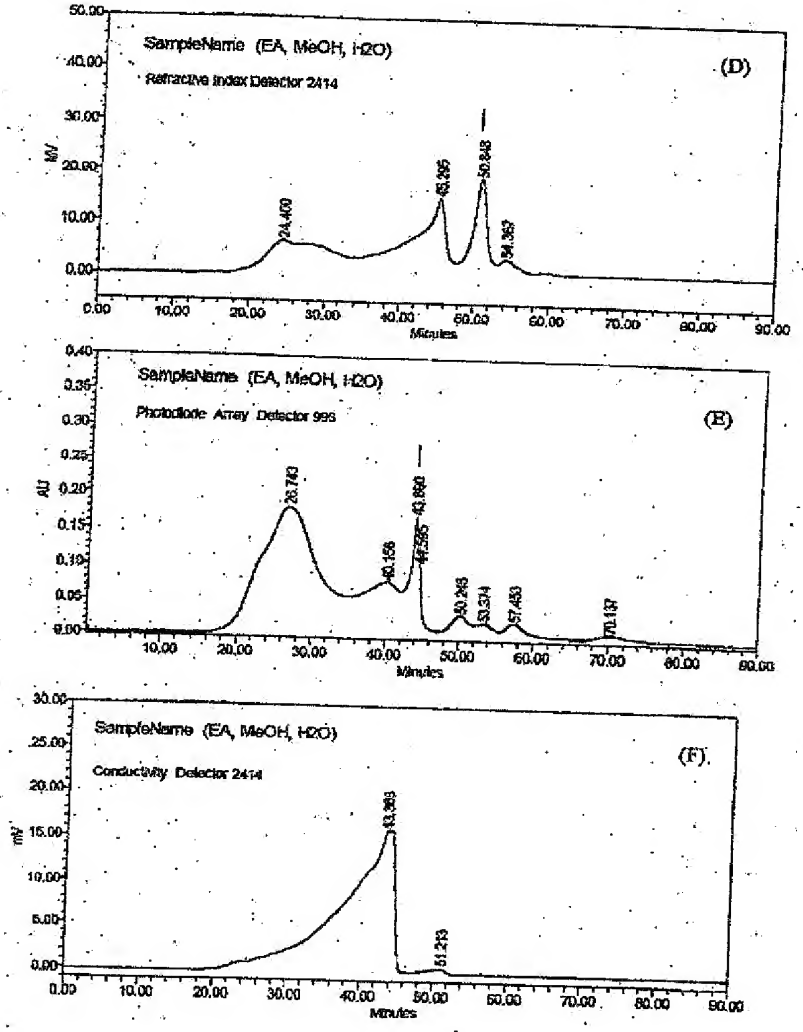

(ether, MeOH, H2O)
(A)
Refractive Index Detector 2414
mV
Minutes
23.883
29.683
44.017
45.632
50.937
54.469
(ether, MeOH, H2O)
(B)
Photodiode Array Detector 996
AU
Minutes
26.863
40.799
44.391
50.508
57.648
70.373
(ether, MeOH, H2O)
(C)
Conductivity Detector 2414
mV
Minutes
23.242
42.439
43.219
51.099

Figure S2 (continued)






5. I declare under penalty of perjury that I have performed the above-identified experiment and that the information contained herein is true and correct.

Executed at Taipei, Taiwan, R.O.C. on September 13, 2006.

2006-9-13